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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 09/058,323 | 04/09/1998 | BEREND HOUWEN | 10690/101683 | 7347 |

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BRYAN CAVE
245 PARK AVENUE
NEW YORK, NY 101670034

| EXAMINER |
|---|
| GABEL, GAILENE |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1641 | 24 |

DATE MAILED: 04/02/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. 09/058,323 | Applicant(s) HOUWEN ET AL. | |
|---|---|---|---|
| | Examiner Gailene R. Gabel | Art Unit 1641 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *24 January 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

## DETAILED ACTION

### *Amendment Entry*

1. Applicant's response filed 1/24/02 in Paper No. 23 is acknowledged and has been entered. Currently, claims 1-13 are pending and under examination.

### ***Rejections Maintained***

### *Claim Rejections - 35 USC § 102103*

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. Claims 1-3 and 5-9 stand rejected under 35 U.S.C. 102(e) as being clearly anticipated by Kim et al. (US 5,648,225) for reason of record.

3. Claims 4 and 10-13 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Inami et al. (US 5,298,426) for reason of record.

***Response to Arguments***

4. Applicant's arguments filed 1/24/02 have been fully considered but they are not persuasive.

A) Applicant argues that Kim only discloses a multipurpose reagent system having salts and buffers, and including nuclear stain and antibody against cell surface antigens. Applicant argues that Kim fails to teach, suggest, or disclose all the elements of the claimed invention in failing to disclose use of a nucleotide fluorescent dye and a fluorescent binding antibody in the same method or process as the claimed invention. Applicant then argues that the two particular steps of staining as claimed by Applicant are missing from Kim.

Contrary to Applicant's argument, Kim does, teaches the method as claim by Applicant. Specifically, Kim teaches how the multipurpose reagent system is used in a method of discriminating and counting between leucocytes and erythroblasts or nRBCs. Kim teaches adding 1) fluorochrome-conjugated antibodies directed to leucocyte surface antigens, 2) nucleotide fluorescent dye, i.e. ethidium homodimer, and 3) a proper concentration of aldehydes, salts, and buffer, in a multipurpose reagent system, to an anticoagulated blood sample, incubating the mixture, and subjecting the mixture to flow cytometric analysis. The fluorochrome-conjugated antibodies directed to leucocyte

surface antigens bind and stain leucocytes. The nucleotide fluorescent dye stains the exposed nuclei of erythroblasts, but does not penetrate the intact white cells, thus allowing quantitative analysis of nucleated red cells. The mixture of aldehydes, non-quaternary mono-ammonium salt, and buffer permeablizes, i.e. lyses, the erythroblasts while maintaining the integrity of the fixed white blood cells. Electronic signals from scattered light collected from different angles and fluorescence intensities are plotted as two- dimensional plots in column 6, lines 31-46 and also Figure 3. Given the teaching of Kim et al. wherein all the required elements are disclosed for the same use or purpose in flow cytometry, it is maintained that claims 1-3 and 5-9 are anticipated by Kim because as such, no patentable distinction is seen.

B) Applicant argues that Kim does not teach simultaneous analysis of a sample using erythroblast nucleotide dye staining and leucocyte cell surface marker fluorescent labeling as demonstrated by Applicant by simultaneous analysis of their signals.

Contrary to Applicant's argument, Kim does, teach simultaneous analysis of a sample using fluorochrome-conjugated antibodies directed to leucocyte surface antigens and nucleotide fluorescent dye in a multipurpose reagent mixture as previously discussed. Alternatively, the feature upon which applicant further relies (i.e., simultaneous analysis) is not distinctly recited in the rejected claims.

C) Applicant argues that the combination of the teaching of Kim and Inami does not suggest the affirmative and manipulative steps taken with respect to the two signals

appearing in Applicant's claims such as detecting, analyzing, and discriminating. Applicant also argues that there is no suggestion and motivation to combine the teaching of Kim and Inami and that Examiner's explanation of the motivation supporting the combination underlying the rejection is insufficient.

In response, the affirmative and manipulative steps taken with respect to the fluorescent signals such as detecting, analyzing, and discriminating are clearly inherent considering the analogous use of flow cytometric analysis by both of Kim and Inami just as claimed by the instant invention. Kim teaches combining 1) fluorochrome-conjugated antibodies directed to leucocyte surface antigens, 2) nucleotide fluorescent dye, and 3) a proper concentration of aldehydes, salts, and buffer, in a multipurpose reagent system, in order to simultaneously quantitate and discriminate between leucocytes and nucleated RBCs using flow cytometric analysis. Inami is incorporated for the disclosed two reagent system to substitute for the buffer solution taught by Kim so that the erythroblasts are not lysed, but rather, their cell membranes are increased for permeability of a nucleotide fluorescent dye. Inami discloses a 1) hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic range, and 2) a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained. The first acidic and hypotonic fluid has a low osmolality causing erythrocytic cell lines in the sample to swell upon absorbing water causing cellular contents to leak out and nucleotide fluorescent dye (erythroblastic) dye

to diffuse through the cell membrane to stain their nuclei while leucocytes do not permit the entrance of nucleotide fluorescent dye. Inami discloses that the concentration of propidium iodide or ethidium bromide, should fall within the range of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) in order to achieve optimum results in flow cytometric analysis. Given the combined teaching of both Kim and Inami, wherein appropriate reagents and concentrations thereof are used, all of detection, analysis, and discrimination between desired populations using measurements of fluorescence intensities or fluorescent signals, manipulation of results obtained, and population distribution in a histogram are precisely the power of flow cytometric analysis.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to substitute the buffer solution of Kim with the two reagent system taught by Inami for use in permeabilizing erythroblasts because Kim specifically taught that integrity and antigenicity of white blood cells need to be optimally maintained during permeabilization, i.e. lysing, of the nRBC's or erythroblasts in order to allow accurate simultaneous quantitation of both populations and Inami specifically taught that the two reagent system eliminates lysing conditions for erythroblasts while maintaining the integrity and shape of WBCs for accurate differentiation of both erythroblast and leucocyte populations.

Further, absent evidence to the contrary, the nucleotide fluorescent dye disclosed by Inami in the concentration of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) would have been able to effectively perform the same erythroblast maturity

differentiation and quantitation as set forth in instant claims 11-12 upon subjecting the sample mixture to flow cytometry .

5. For reasons aforementioned, no claims are allowed.

6. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R. Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday, 6:30 AM - 4:00 PM and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (703) 308-3399. The fax phone numbers for the

organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Gailene R. Gabel
March 28, 2002

CHRISTOPHER L. CHIN
PRIMARY EXAMINER
GROUP 1800 1641